respectively. The presence of high-order, fluorescently-labeled Gag oligomers at the membrane can impact fluorescence monitoring of the cytoplasmic fraction of Gag. In addition, cytoplasmic species can confuse the study of membrane-bound Gag complexes. Our work focuses on the use of fluorescence fluctuation spectroscopy (FFS) and total internal reflection fluorescence (TIRF) microscopy to investigate retroviral Gag species, both at the membrane and in the cytoplasm. A focus of the study is the relationship between cytoplasmic Gag concentration and the formation of Gag assembly sites at the plasma membrane. FFS monitors Gag cytoplasmic concentration, while TIRF monitors the biogenesis of HTLV-1 virus-like particles. Comparison of results from HTLV-1 and HIV-1 Gag reveals intriguing differences in their assembly pathways. This work is supported by NIH Grant AI81673, NIH Grant GM064589, a Cancer Center Cancer Biology Training Grant (T32CA09138), and an American Cancer Society Postdoctoral Fellowship (PF-11-159-01-MPC).

1047-Pos Board B833

Fluorescence Fluctuation Spectroscopy Applied to Cell-Free Expression, Chromophore Maturation and Protein-DNA Interaction

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Cell-free expression systems are increasingly being employed as platforms for biophysical, biochemical, and systems biology experiments. We demonstrate that combining fluorescence fluctuation spectroscopy (FFS) with cell-free expression provides quick and quantitative measurements of chromophore maturation, protein-protein interactions and protein-DNA interactions. We perform the first study of chromophore maturation as a function of temperature, and demonstrate pronounced temperature dependence of the maturation kinetics for EGFP, EYFP, and mCherry. The Eyring equation successfully reproduces the temperature-dependence of the maturation rate for each of the proteins. Our results for EGFP, EYFP and mCherry provide an explanation for the differences in the reported maturation times studied by de novo protein synthesis. A droplet sample protocol was developed to ensure sufficient oxygenation for chromophore maturation studies, while preventing evaporation of the sample. We further demonstrate the feasibility of protein titrations with the droplet protocol and characterize oligomerization of the nuclear transport factor 2 (NTF2) over a wide concentration range by brightness analysis. We employ the droplet setup to study APOBEC3G, a DNA cytosine deaminase enzyme with innate immune activity against retroviruses, notably HIV-1. Brightness analysis reports the homo-oligomerization of APOBEC3G, which is both concentration and temperature dependent. Using two-color FFS, we simultaneously measure APOBEC3G oligomerization and the interaction between APOBEC3G and single-stranded DNA (ssDNA) in order to characterize the molecular interactions of this important enzyme. This work was supported by grants from the National Institutes of Health (GM64589, GM091743).

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Blinking and Bleaching of Tetramethylrhodamine on DNA Induced by Paramagnetic Cations

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Certain proteins require metal cation cofactors to function. The conformations and dynamics of such proteins are often studied using single molecule fluorescence techniques. Blinking of fluorophores can make analysis of single molecule fluorescence data difficult. During the investigation of a manganese-dependent enzyme that binds DNA, we observed fluorescence fluctuations of tetramethylrhodamine (TAMRA) when in the presence of manganese, but not magnesium. This project seeks to understand the origin of fluorescent fluctuations when in presence of certain cations and why they occur.

A DNA strand with an internally labeled TAMRA was studied while in the presence of divalent calcium, magnesium, manganese, cobalt, nickel, and zinc. Using fluorescence correlation spectroscopy, the fluctuations of TAMRA were analyzed. When in the presence of calcium, magnesium, and zinc, the correlation of TAMRA can be described by diffusion only. However, a process that occurs in the micro-second timescale is present when manganese, cobalt, and nickel are present. Flash photolysis experiments were done to characterize the nature of the excited state species that causes fluorescence fluctuations. The results indicate that a triplet forms while in the presence of manganese but not magnesium, and only in the presence of DNA. Manganese, cobalt, and nickel are paramagnetic cations and can induce triplet formation in dyes, while diamagnetic cations, like calcium, magnesium, and zinc, do not. This conclusion is supported through lifetime and photodegradation experiments. When DNA is present, the cations can induce a triplet at much lower concentrations than when DNA is not present. This can be explained by the fact that when cations bind to DNA, they are in closer proximity to the dye.

1049-Pos Board B835

Single Molecule Fluorescence Dynamics and On/Off Blinking of Photoactivatable Fluorescent Protein mEos2

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Blinking, the natural switching of a fluorophore between fluorescent and dark states, has been well documented for commonly used fluorescent proteins such as GFP and YFP. However, blinking behaviors of photoactivatable fluorescent proteins popular in superresolution imaging have not been well characterized. Blinking fluorophores can be exploited to obtain subdiffraction-limited resolution, but counting a single fluorophore multiple times could lead to misidentification of structural features and difficulties in quantifying constituents of cellular structures. We describe the blinking behavior and related photophysical, photochemical properties of mEos2, the most widely used photoactivatable fluorescent protein in super resolution imaging. We analyze variations of single mEos2 molecules in fluorescence emission, lifetime, and blinking rates and their dependence on the power of activation and excitation energy. These results are interpreted and utilized in the context of obtaining superior superresolution images.

1050-Pos Board B836

Enhancing Temperature and Pressure Sensitivity of Various Fluorescent Proteins

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Green fluorescent protein (GFP), a spontaneous fluorescent protein isolated from Pacific jellyfish, is perhaps the most popular fluorescent probe due to its simple and easy labeling procedure. Using GFP as a prototype, many fluorescent protein variants of different wavelength emissions have been developed by gene engineering. These can be used as indicators for numerous physiological properties like pH, Ca2+ concentration, and force to study the dynamic states of a living cell. Here we exploit the dependence of wavelength emissions on temperature and pressure to develop a fluorescent protein- pressure indicator.

We investigated the temperature and pressure dependencies of Cyan fluorescent protein (CFP), GFP, and Yellow fluorescent protein (YFP). For an increase in temperature, the intensities of all three proteins decreased. However, for a pressure increase, the intensities of CFP and GFP increased while that of YFP decreased. We also found a common mutation, an insertion of several glycines into β-strand 7, that enhances the temperature and pressure dependencies of all three proteins, causing a spectra blue shift in YFP, but not in CFP or GFP. In this meeting, we will discuss these temperature and pressure dependencies and the effect of our mutation.

1051-Pos Board B837

Microfluidic Cytometer For Simultaneous High-Throughput Screening of Fluorescent Proteins on the Basis of Fluorescence Lifetime, Photostability, and Brightness

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Although the excitation/emission spectra of red fluorescent proteins (RFPs) have been ideally tuned to a window optically favorable for in vivo work, their quantum yields, photostabilities and blinking properties require improvement if they are to be broadly employed for low-copy or single-molecule measurements. Attempts to engineer improved RFPs often result in optimization of one photophysical property at the expense of others. We developed a microfluidic-based cytometer for screening HeLa cell-based genetic RFP-libraries simultaneously on the basis of fluorescence lifetime (a proxy for quantum yield), photostability, and brightness. Nine 532 nm excitation beams interrogate each cell in flow. The first is acousto-optically modulated (1-50 MHz) to enable lifetime measurement with phase fluorimetry. The remaining beams act as a pulse sequence for isolating the irreversible photobleaching time constant. A 2D scatter plot is shown below. Optical-force switching is employed to sort cells based on any combination of the photophysical parameters. Screening with this instrument enables identification of regions of the structure that synergistically affect